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THESIS

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**STUDIES ON
THE THYROID HORMONES**

by

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by

JAN-GUSTAF LJUNGGREN

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TO MY PARENTS

STUDIES ON THE THYROID HORMONES

AKADEMISK AVHANDLING

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Jan-Gustaf Ljunggren

med. kand.

This thesis is a summary of the following papers, which will be referred to in the text by the Roman numerals I to V.

- I. Ljunggren, J. G.: The oxidation of 3,5-diiodotyrosine by peroxidase and hydrogen peroxide. *Acta Chem.Scand.* 17 (1963) 567
- II. Ljunggren, J. G.: The isolation and identification of 2,6-diiodohydroquinone from the thyroid gland. *Acta Chem.Scand.* 15 (1961) 1772.
- III. Sörbo, B. and Ljunggren, J. G.: The catalytic effect of peroxidase on the reaction between hydrogen peroxide and certain sulfur compounds. *Acta Chem.Scand.* 12 (1958) 470.
- IV. Ljunggren, J. G. and Sörbo, B.: The donor activity for peroxidase and the effect on the thyroid gland of certain tyrosine derivatives. *Acta Chem.Scand.* 17 (1963) 563.
- V. Bergfelt, G., Ljunggren J. G. and Hedberg, K.: Preoperative treatment of thyrotoxicosis with antithyroid drugs and thyroxine. *J.Clin.Endocrinol.* 21 (1961) 72.



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General introduction

Warton in 1656 suggested that "the function of the thyroid gland is to round and beautify the neck by filling the vacant spaces about the larynx, and particularly in females to whom for this reason a larger gland has been assigned".

This function may of course still be of some importance, but at present the most valuable function of the thyroid gland is believed to be concerned with the production of thyroxine, which acts as a potent stimulus in the rate of cellular oxidation in all the cells of the body.

At the start of this investigation the mechanism of thyroxine biosynthesis was not known. It had been suggested that iodide and tyrosine formed mono- and diiodotyrosine, and that diiodotyrosine in some way formed thyroxine.

That the enzyme peroxidase was involved in the formation of diiodotyrosine from iodide and tyrosine, and perhaps also in the formation of thyroxine was also suggested.

The present investigation was started in order to examine the effect of peroxidase on diiodotyrosine and, if possible, to isolate thyroxine or other products from the reaction mixture.

Antithyroid drugs inhibit the formation of thyroxine, and it has been postulated that these compounds act by interfering with peroxidase activity. It was then of interest to make a further investigation of this inhibitory mechanism. Thus the action of peroxidase on some of the well-known antithyroid drugs was investigated. It was also of interest to investigate if a rapid *in vitro* test for the effect of antithyroid compounds on peroxidase could be elaborated. With the aid of such a test, compounds having no effect on peroxidase could perhaps be excluded as antithyroid drugs, and effective compounds could be further investigated *in vivo*.

During the investigation of these problems, the author came in contact with a surgical clinic in which antithyroid drugs were used in the preoperative treatment of thyrotoxicosis. This contact gave rise to the evaluation and application of a method of preoperative therapy based upon the combined treatment of antithyroid drugs and thyroxine.

The following discussion of these investigations concerning the thyroid gland, will, for practical reasons, be divided into the following three main parts.

Part 1. The participation of peroxidase in the biosynthesis of thyroxine (I to II)

Part 2. The effect of antithyroid agents on peroxidase (III to IV)

Part 3. The effect of thyroxine administration on thyrotoxic patients pretreated with antithyroid drugs (V).

PART 1

The participation of peroxidase in the biosynthesis of thyroxine

Introduction

Ever since Kendall (1, 2) in 1915 succeeded in isolating thyroxine from the thyroid gland and Harington (3), some years later determined its chemical structure, many investigations on its biosynthesis have been carried out.

Many excellent summaries concerning the formation of thyroxine have recently been published (*e.g.* 4, 5) and therefore only some of the more important investigations relevant to this work will be reviewed in this paper.

According to present knowledge, the biosynthesis of thyroxine can be divided into three stages.

1. Concentration of iodide in the thyroid.
2. Oxidation of iodide, and iodination of tyrosine to monoiodotyrosine and diiodotyrosine.
3. Formation of thyroxine from diiodotyrosine.

1. Concentration of iodide in the thyroid

The ability to concentrate iodide from the circulation constitutes the first stage in the fixation of iodide by the thyroid. The ratio between the concentration of iodide in thyroid tissue and serum of normal man is about 50:1 (6). Although much work has been carried out, the mechanism for this accumulation is yet still unknown. It may be possible that some specific protein either in the thyroid cell mass, or cell membrane is involved in the transportation of iodide from the plasma into the gland. Experimental data have shown that this concentration mechanism is specific not only for iodide, but also functions with other ions of comparable ionic size and valency (7a). Thus the protein is believed to have a spatial arrangement that will specifically chelate all monovalent ions which fulfil certain requirements of size, and thus be adaptable into its spatial structure.

2. Oxidation of iodide and iodination of tyrosine to monoiodotyrosine and diiodotyrosine

The iodide which is concentrated by the thyroid must first be oxidized before it can react with tyrosine. Experimental results have been published in the last few years which give strong support to the postulation (8) that peroxidase may catalyze this oxidation process, and perhaps also the synthesis of organically bound iodine.

The presence of peroxidase in the thyroid gland is now well established. The first

indications were reported in 1944 by Dempsey (9) who obtained histochemical evidence of peroxidase activity in thyroid cells. Two years later De Robertis *et al.* (10) described peroxidase activity in both cell and colloid of rat tissue. The enzyme was partly purified from pig thyroid and some properties were investigated by Hosoya *et al.* (11, 12).

The formation of 3-monoiodotyrosine from tyrosine and 3,5-diiodotyrosine from 3-monoiodotyrosine in the presence of iodides, peroxidase, and hydrogen peroxide has been reported (13).

Serif *et al.* (14) reported that hydrogen peroxide was required for the synthesis of 3-monoiodotyrosine from carrier-free I^{131} -iodide and tyrosine by thyroid preparations.

Alexander (15, 7c) demonstrated that the iodide ion is oxidized by hydrogen peroxide and the enzyme iodide peroxidase, before it is incorporated into tyrosine by thyroid homogenate. The results were verified by De Groot *et al.* (16, 17), who also described a soluble thyroidal iodide-peroxidase, tyrosine-iodinase system in the thyroid gland. The catalytic effect of peroxidase on the incorporation of iodine into tyrosine had also recently been described and discussed (18). Thus peroxidase may catalyze both the oxidation of iodide and the iodination of tyrosine.

3. Formation of thyroxine from diiodotyrosine

It is now generally accepted that diiodotyrosine is the precursor of thyroxine in the thyroid gland. Experimental proof of the precursor-product relationship between the compounds has been published (19, 20), the data being obtained by determination of their specific activity at different time intervals.

Although much work has been done, the reaction mechanism for the formation of thyroxine from diiodotyrosine is not yet clear.

In 1926, Harington (3) suggested that thyroxine may be formed from the coupling of two molecules of diiodotyrosine and the loss of an alanine molecule. The first experimental support of thyroxine formation from diiodotyrosine came in 1939 from the classical work of von Mutzenbecher (21), in which he incubated diiodotyrosine at an alkaline pH under aerobic conditions, and small amounts of thyroxine were formed. This reaction was further studied by Johnson and Tewkesbury (22), who not only isolated thyroxine but also pyruvic acid and ammonia. The identification methods for the last two compounds were, however, not reported, and the results have not been confirmed. They also postulated that hypoiodite was the effective oxidizing agent. In 1944 Harington (23) suggested that the alanine moiety lost by one molecule of diiodotyrosine, appeared first as dehydroalanine which in turn would be oxidized to serine or to pyruvic acid and ammonia. The following year Harington and Pitt-Rivers (24), in further studies stated that the reaction was profoundly influenced by the pH (optimum pH 10). The addition of oxidizing agents was investigated, and the results indicated that the reaction was oxidative, aerobic, and was accelerated by iodine and hydrogen peroxide. Examination of the reaction products (25) revealed the formation of thyroxine,

4-hydroxy-3,5-diiodobenzaldehyde, oxalic acid and alanine at high pH. The best net yield of thyroxine in these experiments was about 4 per cent. However, when the amino group of diiodotyrosine was blocked by acetylation, the yield of acetylated thyroxine increased to about 20 per cent. Protection of both the amino and carboxyl groups of diiodotyrosine yielded about 35 per cent of the corresponding thyroxine compound. The nature of the material lost during the reaction could not be identified. In a later work (26) several other diiodotyrosine derivatives were investigated. When α -N-acetyl- ϵ -(N-acetyl-diiodotyrosyl)-lysine was oxidized, 50 per cent net yield of the corresponding thyroxine derivative was formed.

The structural effects in the conversion of analogs without the amino group of diiodotyrosine to analogs of thyroxine has been investigated (27). The results indicated that the formation of the thyroxine analog was very much dependent on the structure. The best yield (11 per cent) was obtained from the propionic acid analog. This analog was further studied (28) and the results indicated that the material lost during the reaction is probably converted to a hydroxylated compound (either serine or hydroxypyruvic acid).

In 1953 Tong *et al.* (29) incubated I^{131} -labelled diiodotyrosine with slices and homogenates prepared from the thyroids of sheep and also from livers and kidneys of rats. The results showed that thyroid slices partly deiodinated diiodotyrosine and incorporated the iodine into thyroglobulin. Thyroid homogenate was only weakly active in deiodinating diiodotyrosine. There was no evidence of any deamination, either in the presence or absence of pyruvate or α -ketoglutarate, and no metabolic products were detected. However, both liver and kidney slices readily metabolized diiodotyrosine in the presence of oxygen and under anaerobic conditions. The principal metabolic products were inorganic iodide, 4-hydroxy-3,5-diiodophenyllactic acid, 4-hydroxy-3,5-diiodophenylpyruvic acid and an unidentified compound. Liver and kidney homogenates had only a slight action on diiodotyrosine, but when pyruvate or α -ketoglutarate were added these homogenates were quite as active as slices in metabolizing diiodotyrosine, both aerobically and anaerobically. The principal reaction products were the unknown compound and 4-hydroxy-3,5-diiodophenylpyruvic acid. However, Horvath (30) recently has shown that mitochondria from the thyroid gland of dogs in fact transaminates diiodotyrosine in the presence of α -ketoglutarate, producing p-hydroxydiiodophenylpyruvate and glutamic acid.

Tong *et al.* (29) also found in the same investigation that diiodotyrosine was readily metabolized by rattlesnake venom which contains an 1-amino oxidase. The principal reaction products were 4-hydroxy-3,5-diiodophenylpyruvic acid, the unknown compound and inorganic iodide. The oxidation of diiodotyrosine with 1-amino oxidase has also been investigated by others (31, 32), and the formation of 4-hydroxy-3,5-diiodophenylpyruvic acid was confirmed. The formation of 4-hydroxy-3-iodophenylpyruvic acid (31, 32) and 4-hydroxy-3,5-diiodophenylacetic acid were also described (32).

Hillmann (33) found that 3 per cent of thyroxine was formed when 4-hydroxy-3,5-diiodophenylpyruvic acid reacted with either diiodotyrosine or N-acetyl-diiodotyrosine. The formation of thyroxine from 4-hydroxy-3,5-diiodophenylpyruvic acid and diiodotyrosine in the presence of an oxidizing agent, has been described by other investigators (34, 32). The yield of thyroxine was about 25 per cent at pH 7.6. The incubation of N-acetyl-diiodotyrosine under similar conditions produced N-acetyl-thyroxine. That peptides of diiodotyrosine can couple with 4-hydroxy-3,5-diiodophenylpyruvic acid, in a similar manner as diiodotyrosine itself, to form peptides of thyroxine has also been demonstrated (35).

The conversion of diiodotyrosine to thyroxine by rattlesnake venom in presence of oxygen and of catalase has recently been described (35, 36). The yield of thyroxine was about 12 to 16 per cent at pH 6.7.

The presence of the 3-mono- and 3,5-diiododerivatives of 4-hydroxyphenylpyruvic acid in rat thyroid gland has been demonstrated (37). However, 4-hydroxy-3,5-diiodophenylpyruvic acid was identified employing an alkaline paper-chromatographic system where the compound is easily converted to the corresponding benzaldehyde (33), and both compounds have the same R_f value in the system used (I). Therefore, as pointed out in Paper I, reservation must be made for the identification procedure.

Personal preliminary experiments (38) indicated that diiodotyrosine was oxidized by myeloperoxidase and hydrogen peroxide. Myeloperoxidase was used, as this enzyme originates from animals and was thus believed to be more similar to the peroxidase of the thyroid gland than that of plants. It is worth noting that when these experiments were started in 1957, the presence of peroxidase in the thyroid gland had not been finally established. In these oxidation experiments 3,5-diiodobenzoquinone could be isolated and identified as reaction product, which was reduced to the corresponding hydroquinone, and a semi quantitative determination of the amount of the hydroquinone revealed the total yield of the compound to be about 20 per cent of the theoretical value. The yield was calculated on the initial amount of diiodotyrosine. On analysis of the reaction mixture by paperchromatography employing a butanol:acetic acid:water (4:1:1) solvent system, a ninhydrin positive spot was found with the same R_f value as alanine. However, further studies (I) showed the compound not to be alanine, and no further investigations were carried out.

Personal, unpublished, preliminary results further showed that incubation of I¹³¹-labelled diiodotyrosine and diiodohydroquinone in phosphate buffer, pH 7.4, lead to the formation of I¹³¹-labelled thyroxine. The yield was a few per cent. Experiments were also performed with the addition of thyroid homogenate. Thereby, the yield of I¹³¹-labelled thyroxine increased but control experiments revealed that a transiodination process between the added I¹³¹-labelled diiodotyrosine and thyroxine, present in the homogenate, was responsible for the major part of the yield increase. This reaction will be investigated further.

The formation of thyroxine from diiodotyrosine and diiodohydroquinone in about one per cent yield has been reported previously by Lissitzky *et al.* (39). The presence of diiodohydroquinone, or a related compound, in the thyroid gland under certain conditions was also reported in the same investigation. However, no prescription of the isolation or identification procedures was given.

It is not yet known if thyroxine is formed *in vivo* from free or protein-bound diiodotyrosine. Experimental data mostly favour the latter postulation (40). It is also in agreement with *in vitro* experiments where the yield of thyroxine from diiodotyrosine is higher if the amino or carboxyl groups is blocked.

Triiodothyronine is also formed in the thyroid gland by two main pathways being postulated (4a): The first consists of a coupling somewhat similar to that involved in the formation of thyroxine, in which one molecule of monoiodotyrosine and one molecule of diiodotyrosine participate, and the second consists of a partial deiodination of thyroxine.

The present investigation

As described above, peroxidase has been associated with the formation of diiodotyrosine from iodide and tyrosine. The present investigation was undertaken in order to examine the action of peroxidase on diiodotyrosine. Preliminary results showed that 3,5-diiodobenzoquinone could be isolated as a reaction product, and it was thus of great interest to make further detailed studies on the reaction mechanism, and if possible to investigate if the quinone was present in the thyroid gland.

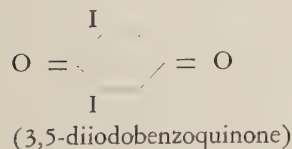
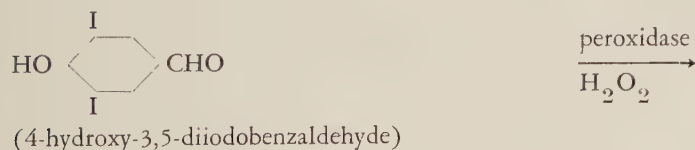
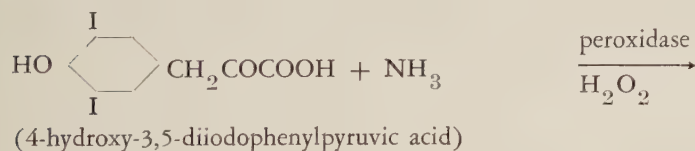
The results of the oxidation studies are reported in Paper I. In this investigation horseradish peroxidase was used in order to examine if any specificity differences between this and myeloperoxidase on the action of diiodotyrosine could be found.

Diiodotyrosine labelled with I^{131} was oxidized with horseradish peroxidase and hydrogen peroxide (I). The reaction mixture was first analyzed by column chromatography according to Spackman *et al.* (41). The results showed that alanine, serine and tyrosine were absent. Monoiodotyrosine and ammonia were found as reaction products, and ammonia was also quantitatively determined employing Nessler's reagent. When the reaction mixture was investigated by radiochromatography, inorganic iodide, 4-hydroxy-3,5-diiodophenylpyruvic acid and 4-hydroxy-3,5-diiodobenzaldehyde could be identified. In the solvent systems used for this identification, 3,5-diiodobenzoquinone did not move and thus could not be unambiguously identified. Therefore the presence of the compound was verified in the following manner. According to difficulties in chromatographing the benzoquinone, it was first reduced to the corresponding hydroquinone and then identified by radiochromatography. 4-hydroxy-3,5-diiodophenylpyruvic acid was also oxidized with horseradish peroxidase and hydrogen peroxide. 4-hydroxy-3,5-diiodobenzaldehyde and 3,5-diiodobenzquinone, identified as 3,5-diiodohydroquinone, were found. The absence of tyrosine in the reaction mixture does not exclude the pos-

sibilities of the compound being formed, as tyrosine is also a substrate for peroxidase. The oxidation products of tyrosine have recently been investigated (42). The main product is dityrosine. If the deiodination occurs *in vivo* it is possible that the lost iodine is incorporated into a new tyrosine molecule. (Further, unpublished, results showed that incubation of diiodotyrosine with myeloperoxidase and hydrogen peroxide gave similar reaction products as in experiments with horseradish peroxidase. The compounds being identified by radiochromatography).

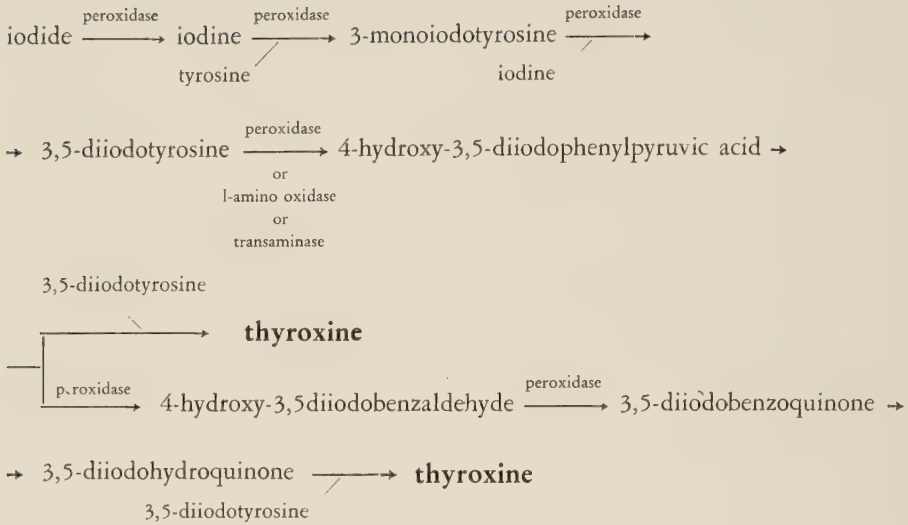
It was of interest in this connection to investigate if 3,5-diiodobenzoquinone is present in the thyroid gland. The results of this investigations are reported in Paper II. Due to the above mentioned difficulties in chromatographing 3,5-diiodobenzoquinone it was decided to reduce the compound to the corresponding hydroquinone before the isolation procedure started. After administration of I^{131} to rabbits, I^{131} -labelled 3,5-diiodohydroquinone could be isolated from the thyroid glands. The compound was identified by radiochromatography. Five new solvent systems for the compound were worked out. The identity was further established by reversed isotope dilution.

Thus, the present investigation suggests the following reaction mechanism:



All the different compounds have been shown to be present in the thyroid gland. Thus, thyroxine may be formed according to Fig. 1.

Fig. 1



It is not known whether the *in vivo* formation of 4-hydroxy-3,5-diiodophenylpyruvic acid from 3,5-diiodotyrosine is catalyzed by peroxidase, 1-amino oxidase or transaminase. These enzymes can catalyze the formation of the keto acid but only peroxidase can also catalyze the formation of the quinone, and the presence of the quinone in the thyroid has been demonstrated. This may favour the peroxidase catalyzed reaction.

PART 2

The effect of antithyroid agents on peroxidase

Introduction

Compounds which inhibit the formation of thyroxine in the thyroid gland may be divided into two groups. The first group includes compounds such as perchlorate which interfere with the iodide-concentrating mechanism, and those of the second group are called "antithyroid compounds", whose inhibitory effect is directed towards the incorporation of inorganic iodide in the tyrosine molecule, or with the formation of other precursors of thyroxine. The second group contains most of the compounds generally employed in the inhibition of the thyroid gland.

Many investigations have been carried out in an effort to find compounds with antithyroid activity, and more than one thousand different substances have been tested. Many excellent reviews (*e.g.* 43a, 44, 45a) concerning these investigations have been published, and therefore this report will only briefly describe some of the more important chemical types of antithyroid compounds.

The first types found to be effective were derivatives of either thiourea or aniline. Later they were extended to include compounds containing the structure $=N-C(=S)-X$ where X was N, C, or S. The compounds most commonly used clinically, at present, from this group are the thiouracil derivatives and the substituted mercaptoimidazoles (45a).

The clinical observation that local administration of resorcinol to patients with skin ulcerations induced hypothyroidism (46) aroused the interest in aromatic compounds. The essential chemical grouping is not yet clearly defined. Derivatives of phenol have been found to be active when appropriately substituted. Substitution in the meta position seems to be favourable. However, many of these compounds are poorly resorbed when administered orally (45a).

The inhibitory mechanism is not yet clear, although there is strong evidence that antithyroid compounds inhibit the formation of thyroxine by interfering with the peroxidase-hydrogen peroxide system. The reason for the postulation is the action of the oxidation system on the biosynthesis of thyroxine, as described previously in the first part of this review. All effective antithyroid compounds so far investigated interfere with the system, and some drugs, *e.g.* thiourea, can be oxidized by peroxidase, and also by living thyroid tissue (44).

It has further been suggested that antithyroid compounds act via the sulfphydryl group

by maintaining the iodine of the thyroid gland in the reduced state, thereby preventing the iodination of tyrosine. There are two major objections to this simple explanation (4b): If thiouracil and related compounds merely act by maintaining iodine in the reduced state, their action should be overcome by administration of sufficiently large amounts of iodide. This mechanism does not account for the goitrogenic action of the sulfonamides or resorcinol.

Some investigations concerning the relationship between biological potency and the effect upon an *in vitro* system of peroxidase and hydrogen peroxide have been reported. However, no real valuable *in vitro* system has been worked out. Randall (47) followed the reaction between horseradish peroxidase, hydrogen peroxide, and the sulfur compounds by a manometric method, which was based on the conversion of the remaining hydrogen peroxide to oxygen through the action of catalase. However, as pointed out in Paper III, the inhibitory effect of the sulfur compounds on catalase (48, 49) was apparently not considered. Rosenberg (50) modified Randall's method and used an enzyme prepared from raw milk. Rimington (7b) showed that no relation existed between antithyroid activity and the effect upon an *in vitro* system of horseradish peroxidase, hydrogen peroxide, and pyrogallol to which different antithyroid drugs were added. However, as pointed out in Paper III, many differences in substrate specificity exist between horseradish peroxidase and myeloperoxidase, and the use of horseradish peroxidase in such a test is very questionable.

To test a compound for antithyroid activity *in vivo* is no easy task, as there are a great many specific variations in the response to these compounds. In man for example, certain substances may show high activity, while in the rat they are of low potency, or vice versa (45a). The stability of other compounds in the gastrointestinal system may vary, of following administration by injection, the initial concentration may be somewhat reduced due to metabolic processes, and therefore does not reach the gland in a sufficiently effective concentration. There might also be limitation in dosage because of toxicity.

The most common methods employed in testing the effect of compounds on the thyroid gland are those measuring the uptake of radioactive iodide at different time intervals. Sometime this method is combined with histological examination of the gland, determination of the amount of free and protein-bound I^{131} , and the distribution of I^{131} among the different thyroxine precursors. The weight of the gland may also be of importance. The determination of the effect on the metabolic rate has also been used but this method seems to be more difficult to perform and is found to be less sensitive than the others (43a).

The present investigation

As described above, the mode of action of antithyroid compounds in the intact animal is probably due to interference with the peroxidase-hydrogen peroxide system.

The present work was carried out in order to investigate the following:

1. The effect of this oxidation system on some of the sulfur containing antithyroid compounds.
2. The specificity variations between myelo- and horseradish peroxidase for these compounds.
3. The possibility of finding a simple method for testing the effect of antithyroid drugs on the peroxidase-hydrogen peroxide system. Substances having no effect on this system could then be excluded as antithyroid drugs, and those substances having a good effect could be further investigated *in vivo*.
4. The possibilities of using such a test in the determinations of new antithyroid compounds.

The first investigation undertaken was the catalytic effect of myelo- and horseradish peroxidase on the reaction between hydrogen peroxide and certain sulfur compounds (III). The compounds investigated were thiourea, thiouracil, thiocyanat, and the "true" thiol compound cysteine, thioglycolic acid and mercaptoethanol. The oxidation of the different compounds were followed in a spectrophotometer at different wave lengths. Myeloperoxidase was found to catalyze the oxidation of thiourea, thiouracil, and thiocyanate, but was inactive with the true thiol compounds. Thiourea was oxidized to formamidine disulfide, formamidine sulfinic acid, sulfate, and an unknown intermediate compound. The identity of the unknown compound was discussed. Myeloperoxidase was also active with thiouracil; the reaction products were not identified. When thiocyanate was oxidized the formation of sulfate and cyanide could be demonstrated together with an unstable intermediate. Horseradish peroxidase was, rather unexpectedly, found to be inactive with all the afore-mentioned sulfur compounds.

A method for testing the effect of compounds on the peroxidase-hydrogen peroxide system was worked out (IV). The method being based upon the amperometric determination of the remaining hydrogen peroxide during the reaction with a rotating platinum electrode.

The donor activity for myeloperoxidase and the effect on the thyroid gland were compared with a new group of substances, all being derivatives of tyrosine. The first compounds analyzed were n-alkylesters of tyrosine, and it was found that the ability of those compounds to function as donors for myeloperoxidase was better for the esters than for tyrosine itself. The ability increased with increasing length of the alkyl chain with a maximum for the butyl — through hexyl — compounds. The ethyl- and pentyl-esters were also assayed for antithyroid activity and were found to be inactive. The reason for this was discussed.

The next group tested contained some other tyrosine derivatives. Among the different compounds, tyramine and its dimethyl derivative were found to be the most active donors

for peroxidase. The effect of tyramine on the thyroid gland was not investigated as this compound has a strong vasopressor action. The other compounds mentioned above were tested for antithyroid activity and found to be inactive. However, dimethyltyramine depressed I^{131} uptake in the thyroid gland, measured after 4 hours, but daily administration to rats for 25 days had no effect. The negative results were discussed. It is possible that the investigated compounds did not enter the thyroid gland in a sufficiently effective concentration.

PART 3

The effect of thyroxine administration on thyrotoxic patients pretreated with antithyroid drugs

Introduction

In the previous chapter, the inhibition of thyroxine formation by antithyroid drugs was discussed. These drugs have for the past 15 years been used in hospitals in the treatment of patients with thyrotoxicosis. When these drugs are used alone in the pre-operative treatment of thyrotoxicosis, the thyroid gland becomes more vascular and friable, thereby increasing the technical difficulties during surgery, and causing a higher incidence of complications. To avoid this, during the last two or three weeks pre-operatively iodide is added to the therapy. With the use of antithyroid drugs, the risk of surgery in less specialized clinics have decreased very significantly, when compared to the risks using iodine alone (45b).

Under normal conditions, the thyroid gland is stimulated by TSH (thyroid stimulating hormone, thyrotrophin) from the pituitary gland. In 1958 Adams (51) reported that the thyroid stimulating agent in the sera of hyperthyroid patients differs from normal TSH in exerting a prolonged secretory stimulation on the thyroid gland, and that administration of thyroxine to a thyrotoxic patient does not decrease the amount of abnormal TSH in the serum.

During the last years many investigations concerning this abnormal TSH have been published and reviewed (*e.g.* 45c, d, 52, 59). According to present knowledge, the abnormal TSH can be divided into two components. The first one is called LATS (long-acting thyroid stimulator) and the second SATS (short-acting thyroid stimulator). The site of production for LATS and SATS in the body is still unknown. LATS and SATS differ from the normal TSH in not being neutralized by an anti-serum of TSH (53, 54, 58). They also differ in not being extractable with alcohol (55, 54). The half-life in the blood for LATS is about $7\frac{1}{2}$ hours and for TSH about $\frac{1}{2}$ hour (56). The maximum effect of TSH and SATS on the animal thyroid gland, pretreated with I^{131} and thyroxine (57), is obtained after about 2 hours, and for LATS after about 9—16 hours (54, 51). The duration of the effect of LATS is longer than that of TSH and SATS (54, 51).

The present investigation

It was of interest to investigate if the enlargement of the thyroid gland, after administration of antithyroid drugs, could be prevented by thyroxine. This enlargement

could be expected to be inhibited by thyroxine if it was caused by an increase of normal TSH.

The present investigation was started in January 1958, before Adams (51) reported his results.

Methyl-mercaptoimidazol was used as the antithyroid drug and administered to the patients until they became euthyroid. At that time, thyroxine was incorporated in the treatment during the last two weeks before the operation. Thus, during continued treatment with antithyroid drugs, the patients were kept euthyroid by the thyroxine administration. The patients were admitted to hospital the day before operation, and were hospitalized for about five to six days post-operatively. The operation was carried out according to standard procedure. Three to four grams of each lateral lobe of the thyroid remained intact.

To obtain uniform material, only the results in those cases of toxic diffuse goiter were reported. The results from this investigation were compared to the results from patients being pretreated by other methods. A comparison was also made with the results from patients suffering from nontoxic goiter in whom the surgical procedure was similar to that of the other groups. The results were discussed with respect to the weight of the removed thyroid tissue, initial BMR, age, body temperature, pulse, and the histological picture of the gland. The weight of the removed thyroid was fairly representative to the size of the gland, as the amount remaining after surgery was the same in each patient.

The patients were divided in 5 groups according to pre-operative medication:

Group A Pretreated with iodine, 237 patients;

Group B Pretreated with antithyroid drug plus iodine, 121 patients;

Group C Pretreated with antithyroid drug plus thyroxine, 47 patients;

Group D Pretreated with antithyroid drug plus iodine and thyroxine, 5 patients;

Group E Control group (nontoxic diffuse goiter) 57 patients.

The following weights of the removed glands for the different groups were found (values given are means $\pm$ standard error):

Group A $37 \pm 2,3$ grams;

Group B $44 \pm 5,8$ grams;

Group C $25 \pm 1,5$ grams;

Group D $37 \pm 5,3$ grams;

Thus the weights were lowest in Group C.

There was no great variation in BMR and temperature between the different groups.

The histological picture varied within each group, but there were marked differences between the groups. In Group A, no glands were devoid of colloid; the colloid content was low in 12 per cent, medium in 52 per cent, and high in 36 per cent. In Group B also, no glands were devoid of colloid; the content was low in 21 per cent, medium in 29 per cent and high in 50 per cent. In Group C the colloid content was nil in 34 per cent of the glands, low in 29 per cent, medium in 32 per cent, and high in 5 per cent. Thus the amount of colloid was lowest in Group C. In Group A the follicles were lined with cuboidal cells in 59 per cent of the glands, cuboidal to low columnar in 38 per cent, and low columnar to high columnar in 3 per cent. In Group B the follicles were lined with cuboidal cells in 28 per cent of the glands, cuboidal to low columnar in 63 per cent, and low columnar to high columnar in 9 per cent. In Group C the follicles were lined with cuboidal cells in 8 per cent of the glands, cuboidal to low columnar in 54 per cent, and low columnar to high columnar in 38 per cent. Thus, the cell-height was lowest in Group A.

No patient experienced any untowards symptoms from the thyroxine medication, either before or after operation.

It would of course have been of interest to determine the TSH concentrations during the treatment, but at the time when these investigations were carried out no suitable method was available.

If sufficient exogenous thyroxine was administered, the cells lining the follicles ought to be cuboidal. In this investigation it is probable that the dosage of thyroxine was too low to inhibit normal TSH completely, as there was no apparent difference between the heights of the follicle cells in Groups B and C. This is also pointed out in the paper.

The pathological examination was performed at the same time for all the investigated glands, and was made as a blind test, i.e. the pathologist did not know the preoperative treatment for the different glands being examined.

The weight of the glands allowed to remain intact was about 6—8 grams. This weight was of course estimated, and based upon the experience of the surgeon (Bergfelt), who has performed more than one thousand variable thyroid operations. Even if the objection can be raised that the surgeon unconsciously allowed more thyroid tissue to remain after the operation for patients in Group C, it is unrealistic to believe that this can account for the low weights obtained in Group C, since this would mean that about 20 grams were allowed to remain instead of about 7.

The investigation shows that it may be possible to prevent the increase in size of the thyroid gland, after treatment with antithyroid drugs, by the administration of thyroxine.

General summary

Diiodotyrosine was oxidized by peroxidase and hydrogen peroxide. The main reaction products were found to be iodide, ammonia, and 3-monoiodotyrosine, together with 4-hydroxy-3,5-diiodophenylpyruvic acid, 4-hydroxy-3,5-diiodobenzaldehyde, and 3,5-diiodobenzoquinone. The presence of 3,5-diiodohydroquinone in the thyroid gland was established. The formation of thyroxine from 3,5-diiodotyrosine and 4-hydroxy-3,5-diiodophenylpyruvic acid (or 3,5-diiodohydroquinone) was discussed.

The action of peroxidase on some of the wellknown antithyroid drugs was investigated. Specificity variations between myelo- and horseradish peroxidase were found. A simple *in vitro* test for the effect of antithyroid compounds on peroxidase was evaluated. Some tyrosine derivatives were investigated in this test system and found to be active, but the compounds were found to be inactive *in vivo* with respect to antithyroid activity. The reason for this was discussed.

A method for pre-operative treatment of thyrotoxicosis was employed and evaluated. The method was based upon a combined treatment of antithyroid drugs and thyroxine. The results were discussed.

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